(12) # EUROPEAN PATENT APPLICATION

(71) Applicant. CONNAUGHT LABORATORIES LIMITED
1755 Steeles Avenue West
Willowdale Ontario M2N 5T8(CA)

(72) Inventor: Hommel, Martin
141 Erskine Avenue
Toronto, Ontario, M4P 1Y9(CA)

(72) Inventor: Sun, Anthony Mein-Fang
4 Barkwood Crescent
Willowdale, Ontario, M2H 3G6(CA)

(72) Inventor: Goosen, Mattheus Florentius Albertus
18 Laxton Avenue
Toronto, Ontario(CA)

(74) Representative: Burford, Anthony Frederick et al,
W.H. Beck, Greener & Co. 7 Stone Buildings Lincoln's Inn
London WC2A 3SZ(GB)

(54) Droplet generation.

(57) Perfectly spherical, smooth and uniform microcapsules, which may contain living cells. are produced having a diameter less than 700 μm by employing an electrostatic droplet generator. A droplet is suspended from a pointed source, such as a needle, and is charged with high static voltage. A collecting vessel or ring device is charged with opposing polarity and attracts the droplet. When a voltage potential threshold is passed, the droplet moves from the source to the collecting vessel. The voltage pulse height, pulse frequency and length, and extrusion rate of the droplet are adjustable so that predetermined sizes of droplets may be repeatedly generated and collected.

FIG.1.
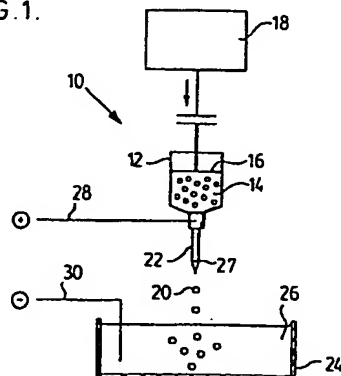
DROPLET GENERATION

The present invention is concerned with droplet
generation, particularly with respect to droplet
generation in the encapsulation of living cells or
individual cells in microcapsules.

Various attempts have been made over the past
twenty years to provide semi-permeable microcapsules
which were both biocompatible with the body tissue and
impermeable to the components of the immune system.
Typical of such attempts is that described in U.S.
Patent Nos. 4,352,883 and 4,391,909 to Franklin Lim.

As set forth therein, living tissue or individual
cells are suspended in an aqueous solution of a
reversibly-gellable material, typically sodium
alginate, and droplets of this suspension are allowed
to drop into a hardening solution, typically calcium
chloride. The temporary capsules so formed are then
treated with polylysine and polyethyleneimine to form
an outer semi-permeable coating. The core material is
reliquified by ion-exchange of the calcium ions.

Survival times of microcapsules produced by this
prior art procedure in the animal body were
consistently less than 3 weeks, thereby severely
limiting the utility of this prior art encapsulation
procedure in the treatment of diseases requiring organ
transplantation, such as diabetes and liver disease.

In our copending European Patent Application
Serial No. 83303354.1 filed June 9, 1983, there is
described an improvement on the above-mentioned prior
art procedure which forms a semi-permeable membrane
which is both biocompatible and yet is able to protect
transplanted tissue and cells from destruction by the
immune system, such that, in animal tests, a single
intraperitoneal transplant of encapsulated islets
reversed the diabetic state for more than one year.

The success of the procedure according to the aforesaid application results from a semi-permeable and durable membrane which has an outer surface of biocompatible negatively-charged material. The improved durability, i.e. resistance to rupture, of these microcapsules is due to their near perfect spherical shape and enhanced capsule membrane thickness.

Although the microcapsules produced in the aforesaid pending application represent a significant advance in the treatment of diseases requiring organ transplantation, there is one drawback which inhibits more ideal utilization of the microcapsules and this drawback arises from the relatively large size of the individual microcapsules, which have a diameter from 700 to 1000 μm. Microcapsules produced according to the procedure of the Lim patents also had relatively large diameters of about 1000 to 2000 μm. Microcapsules having these diameters cannot be injected directly into the cardiovascular system, since they would occlude the blood vessel. Accordingly, the microcapsules must be implanted into large body cavities, such as the intraperitoneal cavity.

Location of the implants in an area of the body other than the cardiovascular system results in an increase in the response time of the microcapsules to changing blood conditions, since the microcapsules are not directly in contact with the blood stream. In addition, the relatively large size of the microcapsules compared to the microencapsulated tissue or cells (e.g. about 200 μm for islets of Langerhans) results in a high diffusional resistance for molecules passing through the microcapsule core.

An air jet-syringe pump extrusion method was used in the procedure of the aforementioned pending application and in the Lim patents to product gel

droplets containing entrapped islets, or other tissue or cells, from the suspension of the islets in aqueous sodium alginate solution. In this procedure, the sodium alginate solution is extruded through a needle located inside a sheathed tube through which air flows at a controlled rate. As liquid droplets are forced out of the end of the needle by the syringe pump, the droplets are pulled off by the shear forces set up by the rapidly-flowing air stream. The higher the volumetric air flow rate, the stronger are the shear forces, the more quickly the droplets are pulled off the end of the needle and the smaller are the resultant droplets.

However, there are inherent restraints in this prior art procedure which prevent the size of microcapsule produced thereby being less than 700 microns. These restraints are that the viscosity of the gel-forming liquid must be greater than 30 cps in order to form perfectly spherical capsules, the minimum internal diameter of the needle must be greater than 300 μm (24 gauge) so as to prevent blockage of the needle by the islets, and the volumetric air flow rate must remain below 2000 cc/min in order to produce capsules of uniform diameter.

We have now discovered an improved procedure and apparatus for forming perfectly spherical, smooth and uniform droplets, such that there can be produced therefrom perfectly spherical, smooth and uniform microcapsules having a diameter of less than 700 μm, preferably about 150 to about 500 μm. Such microcapsules constitute one aspect of the present invention.

The novel microcapsules are formed of biocompatible material and contain living tissue or cells as a core material. A preferred core material is islets of Langerhans, so as to effect long term

control of blood sugar levels in diabetic animals, including humans, by cardiovascular injection of biocompatible microencapsulated islets of Langerhans.

The provision of smaller diameter microcapsules
5   in accordance with this invention, for example 200 to 300 μm, permits direct injection of the microcapsules into the blood stream, so that they may eventually lodge inside body organs, such as the liver or spleen, where they are continuously washed with fresh blood.
10   The direct contact between the microcapsule and the blood significantly decreases the response time of the encapsulated tissue or cells to any biochemical change and thereby increases its efficiency. In addition, the smaller microcapsules result in a lower
15   diffusional resistance for molecules passing through the microcapsule core, further increasing the efficiency of the cells. As a result, fewer of the smaller diameter microencapsulated islets of Langerhans need to be transplanted per kilogram of
20   recipient body weight to achieve prolonged control of blood sugar levels in diabetic patients.

The living tissue-containing microcapsules also may be injected or implanted into any other convenient location in the body to be treated thereby, although
25   this manner of administration is less preferred for the reasons noted above.

The small diameter microcapsules provided in accordance with this invention are formed by employing an electrostatic droplet generator in the initial
30   gel-droplet-forming step. In this procedure, which constitutes a second aspect of the invention, a droplet, suspended from a source, is charged with a high static voltage and a second location, for example, a collecting vessel, is charged with opposite
35   polarity, so as to attract the droplet. When a threshold of voltage difference between the locations is passed, the droplet moves from the source towards

the second location and, thereby, to the collecting vessel.

An adjustable high voltage pulse is generated and applied to the droplet formed on the end of a needle by a syringe pump. The height of the voltage pulse, the pulse frequency and the pulse length are synchronized with the amount of material dispensed, so that known sizes of droplets can be repeatedly generated and collected.

The invention is described further, by way of illustration, with reference to the accompanying drawings in which:

Figure 1 is a schematic representation of a droplet-forming generator constructed in accordance with one embodiment of the invention;

Figure 2 is a schematic representation of a droplet-forming generator, constructed in accordance with a second embodiment of the invention;

Figure 3 is a schematic block diagram representation of the circuitry required to apply electricity to the droplet generator of Figure 1 or 2; and

Figure 4 is a graphical representation of a typical waveform of the output of the droplet generator circuitry of Figure 3.

Referring to the drawings, Figure 1 shows a droplet-forming apparatus 10 constructed in accordance with one embodiment of the invention. As shown therein, a syringe 12 of non-conducting, usually polymeric, material contains a gel droplet-forming liquid 14 which contains living cells. A plunger 16 is driven by a syringe pump 18 to expel droplets 20 from the lower end of a stainless steel syringe needle 22 communicating with the lower end of the syringe reservoir 12, towards a collecting vessel 24 containing a hardening solution 26, which may be aqueous calcium chloride solution in the case of an

aqueous droplet-forming liquid containing sodium alginate.

The positive lead 28 of an electrical pulse generator (see Figure 3) is connected to the needle 22 while the negative lead of the pulse generator is connected to the hardening solution.

The needle 22 may be bevelled at its outlet tip 27, if desired. The tip 27 is located at a specific distance from the top of the recipient medium 26 in the collecting vessel 24 consistent with the voltage pulse to be applied therebetween to effect droplet formation. The size of the droplets 18 may be varied by varying the distance between the needle tip 27 and the liquid in the collecting vessel 24, with shorter distances leading to smaller droplets, by varying the voltage applied by the leads 28 and 30 with increased voltage leading to smaller droplets, by varying the pulse length of applied electricity with decreasing pulse length leading to smaller droplets, or by varying the speed of the pump 18 with decreasing pump speed leading to smaller droplets.

Figure 2 illustrates an alternative arrangement wherein the positive wire 28 from the pulse generator is detached from the needle 22 and instead is attached to a stainless steel ring 32 which is mounted to the lower end of a conical support 34 of non-conductive material which surrounds and extends below the needle 22. The negative lead 30 is attached to the needle 22 rather than to the recipient medium 26. In this arrangement, the distance between the tip 27 of the needle 22 and the top of the recipient medium 26 does not affect the gel droplet size.

The static voltage which is applied by lead wires 28 and 30 during droplet formation in Figures 1 and 2 results in gel droplets having a diameter less than about 700 μm, preferably about 150 to about 500 μm. These droplets then may be coated with a thin coating

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of a semi-permeable biocompatible membrane. The
resulting microcapsules are small enough to be
injected into an animal body using an 18 gauge needle
fitted to a syringe.

5　　　Since the voltage applied during droplet
formation is a static one, the viability of
encapsulated living tissue, such as islets of
Langerhans or liver cells, is not destroyed, and hence
the microencapsulated living tissue is capable of
10　on-going metabolism.

Referring now to Figures 3 and 4 of the drawings,
an electrostatic pulse generator 110 suitable for
formation of electrostatic pulses to be applied during
droplet formation by the apparatus of Figure 1 or 2 is
15　illustrated in Figure 3. As seen therein, the pulse
generator 110 includes an isolated power supply 112,
which may be connected to any desired source of
electric power, logic circuitry 114, console panel 116
having adjusting knobs for pulse frequency 118, pulse
20　width 120 and high voltage output 122, a pulse
amplifier 124, and a high voltage transformer and
rectifier 126 which outputs to the electrical lead
wires 28 and 30.

The electrical pulse voltage, pulse frequency and
25　pulse length which pass to the droplet forming
apparatus 10 by the lead wires 28 and 30 may each vary
widely, depending on the size of droplets desired.
The pulse voltage, which determines the strength of
the force pulling the droplets from the end of the
30　needle 22, usually varies from about 1 to about 25 KV.
The pulse frequency, which determines how many pulses
are applied to the droplet, usually varies from about
10 to about 100 sec^{-1}. The pulse length, which
determines the length of time for which the
35　droplet-forming force is applied, usually varies from
about 1 to about 6 m. sec. The interaction of the
various time periods and their meaning is further

illustrated in Figure 4. These values are synchronized with the amount of material dispensed from the needle to obtain uniformly-sized droplets.

These specific design parameters ensure that
5 there is no voltage overlap since a pulse lasts for 1 to 6 m. sec with each pulse occurring every 10 to 100 m. sec. Accordingly, a minimum of 4 m. sec and a maximum of 99 m. sec occurs between pulses. There is, in addition, a low baseline voltage which maintains
10 the forming droplet in position between pulses.

There are numerous examples in the prior art of devices for electrostatically sorting biological cells, electrostatic sprays for dispensing paints and/or polymers and electrostatic droplet generators
15 for ink printing. Illustrative examples of such devices are described in U.S. Patents Nos. 4,347,935, 4,395,716 and 4,097,373 and British Patent No. 1,346,301. The droplet generators described in these prior patents use an external excitation source, such
20 as acoustic vibration, for the initial formation of the droplet. The droplets are charged electrostatically only after they leave the generator, while in the present invention, the droplet-forming liquid 14 is charged directly by high static voltage.
25 In the prior art arrangements, the external vibrating source causes formation of the droplets while in the present invention droplets are produced by direct electrostatic interaction.

The droplet generator of this invention is
30 capable of producing very small, spherical droplets containing living cells with each step of the droplet formation being under the direct control of the operator.

Although the disclosure herein is directed mainly
35 to the encapsulation of living tissue or cells for the specific purposes and advantages outlined above, it will be understood that the electrostatic droplet

forming method and apparatus described herein has application in other fields and, for example, may be used in spray painting and ink printing.

In this invention, living tissue or individual
5   cells are encapsulated in a biocompatible semi-permeable membrane, in the form of a hydrogel. The material to be encapsulated is suspended in a physiologically-compatible medium containing a water soluble substance which can be reversibly gelled to
10 provide a temporary protective environment for the tissue. The medium is formed into droplets containing the tissue, using the droplet generation procedure of the invention, and gelled, for example, by changing conditions of temperature, pH or ionic environment, to
15 form temporary capsules, of substantially perfect spherical shape. Thereafter, the temporary capsules which result are treated to form a membrane of controlled permeability and negatively-charged outer surface about the shape-retaining temporary capsules.
20 The semi-permeable nature of the membrane permits nutrients and oxygen to flow to the core material and metabolic products to flow therefrom while retaining the core material within the microcapsule. The biocompatible nature of the semi-permeable membrane
25 allows the passage of such materials to and from the core to occur without inflammation or other adverse body response while the outer negatively-charged surface inhibits surficial cell growth, so that the membrane remains semi-permeable and effective for
30 extended periods of time, typically from three to six months or longer.

The temporary capsules may be formed from any non-toxic water-soluble substance that can be gelled to form a shape retaining mass by a change of
35 conditions in the medium in which it is placed, and also comprises plural groups that are readily ionized to form anionic or cationic groups. The presence of

such groups enables surface layers of the capsule to cross-link to produce a permanent membrane when exposed to polymers containing multiple functionalities of the opposite charge.

5   Preferably, the temporary capsules are formed from a polysaccharide gum, which may be natural or synthetic, of a type that can be gelled to form a shape retaining mass by exposure to a change in conditions and can be permanently cross-linked or

10   hardened by polymers containing reactive groups, such as amino groups, which can react with the acidic polysaccharide constituents. Most preferably, the gum is alkali metal alginate, specifically sodium alginate, although other water-soluble gums may be

15   used.

The temporary capsules may be formed from sodium alginate by extruding droplets of aqueous sodium alginate solution into an aqueous calcium chloride solution. It is preferred that the temporary capsules

20   be substantially spherical so that perfectly spherical microcapsules can be formed for cardiovascular injection. Substantially perfectly spherical temporary capsules are formed by using an aqueous sodium alginate solution having a viscosity of at

25   least about 30 centipoise. At viscosities below this critical lower limit, the temporary capsules have an irregular shape. Perfectly spherical capsules are obtained over a wide range of viscosity of the sodium alginate solution above the critical lower limit of 30

30   centipoise, with an upper limit being dictated largely by the ability to extrude the solution into the hardening medium. However, it has also been found that the minimum size of perfectly spherical droplet which can be obtained at a viscosity of at least about

35   30 cps increases with increasing viscosity.

Formation of the permanent semi-permeable membrane about the temporary capsules preferably is

effected by ionic reaction between free acid groups in the surface layer of the gelled gum and biocompatible polymers containing acid-reactive groups, such as, amino groups, typically in a dilute aqueous solution of the selected polymer.

The cross-linking biocompatible polymers which may be used include polyamino acids, preferably polylysine. It is noted that polyethyleneimine and other imine-containing polymers are unsuitable for membrane formation in view of their non-biocompatible nature. The molecular weight of the preferred polylysine polymer should be controlled within a narrow range of about 10,000 to about 30,000, preferably about 17,000, to achieve the required membrane porosity. The use of polylysine or other polyamino acid results in microcapsules having a positively-charged surface, which would be unsuitable for long term viability, although the microcapsules are biocompatible. It is important for long term in vivo life for the polylysine or other polyamino acid to be reacted for a period of time sufficient to develop a substantial thickness of membrane, so as to provide a substantial number of surface groups for post-reaction, as discussed below, sufficient structural strength to permit in vivo injection and sufficient quantity of biocompatible polymer to permit in vivo structural integrity to be retained. Usually, for polylysine of the molecular weight range noted above, a reaction time of at least six minutes is required to achieve these results, generally up to about 9 minutes. These reaction times result in a polylysine layer thickness of about 5 microns.

Surprisingly, the actual strength of the aqueous solution of polylysine used to react with the temporary capsules does not affect the capsule wall thickness, at concentration levels in excess of about 0.05 wt.%.

The semi-permeable membrane formed about the temporary capsules by the reaction with the polyamino acid next is treated with a non-toxic biocompatible water-soluble polymeric material which is capable of ionic reaction with free amino groups to form an outer negatively-charged coating about the membrane, typically by suspension of the microcapsules in an aqueous solution of the polymeric material. The material used to form the outer coating preferably is the same material as is used to form the temporary capsules, preferably a polysaccharide gum, more preferably an alkali metal alginate, such as, sodium alginate. Other biocompatible polymeric materials containing base-reactive groups, such as, polyvinyl alcohol and poly beta-hydroxy butyric acid, may be used to form the outer coating to the microcapsules. Molecular weights of such polymeric materials typically vary from about 10^4 to about 10^6.

The biocompatible water-soluble · polymeric material containing amino-reactive groups reacts with the outer amino-groups of the semi-permeable membrane to form an outer coating. This outer coating permanently shrouds the polyamino acid layer, although leaving intact the porosity of the semi-permeable membrane, and provides a negatively-charged surface. By virtue of the number of surface amino groups on the polyamino acid membrane, resulting from the prolonged reaction time, the outer negatively-charged polymer coating resists degradation and removal, in vivo, so that the positively charged surfaces are not exposed to the body environment.

The treatment of the polyamino microcapsules with the biocompatible base-reactive material retains the overall biocompatible nature of the semi-permeable membrane and results in a negatively-charged outer surface which inhibits cell growth and, therefore, permits the semi-permeable membrane to retain its

permeability and hence effectiveness over an extended
period of time.

Following formation of the microcapsules,
reliquification of the suspending medium for the core
5   material may be effected by re-establishing the
conditions under which the material is liquid. This
may be achieved by ion exchange to remove multivalent
cation, for example, by immersion in phosphate
buffered saline or citrate buffer. The
10 reliquification step, though beneficial in decreasing
diffusion resistance, is not essential for the
provision of an effective product and may be omitted,
since it has been shown that transplanted islets (rat
to mouse) in microcapsules whose interiors have not
15 been reliquified, are also effective in normalizing
blood sugar levels of diabetic animals. Surprisingly,
the calcium alginate gel core does not reliquify
inside the body, since intact gel cores have been
found in microcapsules recovered from diabetic animals
20 up to one year after implantation.

The process of the invention may be used to
encapsulate living tissue, multicellular fractions
thereof or individual cells, for example, islets of
Langerhans, liver cells and red blood cells, and other
25 biologically-active material. The microcapsules which
result may be implanted into an appropriate site
within a mammalian body for the purpose of providing
the body with the specialized physiological function
of the tissue while the tissue remains viable. The
30 implantation may be achieved by simple injection, so
that surgical procedures are not required. As noted
earlier, cardiovascular injection may be effected, in
view of the smaller diameter microcapsules which
result from the electrostatic droplet generation
35 procedure.

The core of the microcapsules contains the living
tissue cells and an aqueous medium of nutrients

sufficient to maintain the tissue and allow its normal metabolism. The cells are viable, physiologically active and capable of ongoing metabolism.

The biocompatible semi-permeable membrane
5   encapsulating the core material consists of interpenetrating layers of ionically-interacted biocompatible materials. The overall wall thickness of the semi-permeable membrane usually varies from about 4 to about 6 µm. The microcapsules themselves
10   have a diameter in the range of less than about 700 µm, preferably in the range of about 150 to about 500 µm for microcapsules containing islets of Langerhans as the core material. The biocompatible semi-permeable membrane is in the form of a hydrogel
15   and hence has an overall water content within the membrane structure of at least about 20 wt%, which may vary up to about 95 wt%, depending on the molecular weight of the polyamino acid.

In a particularly preferred embodiment of the
20   invention, living cells are microencapsulated within a polylysine-alginate semi-permeable hydrogel. The cells are initially suspended uniformly in a sodium alginate solution in physiological saline. Where the microcapsules are to be used for the treatment of
25   diabetes by controlling blood sugar in animals, including humans, the living cells take the form of islets of Langerhans from an animal pancreas.

Spherical droplets containing the cells are produced from an aqueous sodium alginate solution by
30   the electrostatic droplet generation procedure of the invention and are collected as gelled spheres in a hardening solution, such as, calcium chloride. The gelled spheres are coated with polylysine followed by an outer coating of sodium alginate. The
35   microcapsules may then be suspended in isotonic sodium citrate or other convenient ion exchange medium to reliquify the alginate gel inside the microcapsule to

restore the cells to a mobile state. As noted earlier, this step may be omitted, if desired.

The outer biochemically inert but biocompatible alginate surface is a negatively-charged hydrogel 5 containing up to about 95% water. The low interfacial tension between the swollen gel surface and the aqueous biological environment minimizes protein interaction, otherwise a strong protein-polymer interaction may cause a severe inflammatory response. 10 The biocompatibility of the hydrogel membrane leads to long term viability of the capsules when implanted. Polyethyleneimine-surfaced microcapsules do not appear to possess this property, since they produce a strong inflammatory response and hence are rejected by the 15 body, which severely limits the useful in vivo life of the microcapsules. The soft rubbery consistency of most hydrogels may also contribute to their biocompatibility by decreasing frictional irritation to surrounding tissues.

20 The strength of the microcapsules may be increased by additional cross-linking, for example, using glutaraldehyde, prior to reliquification of the gel, if effected.

For in vivo implantation, it is not essential 25 that the biocompatible outer surface be composed of sodium alginate, but it is essential that the outer surface be biocompatible and negatively-charged. Binding occurs between the negatively-charged groups, usually hydroxyl or carboxyl groups, of the 30 biocompatible outer surface material, and the positively-charged amino groups on polylysine.

By the present invention, therefore, there have been obtained biocompatible microcapsules capable of long term in vivo life and having a diameter which 35 render them suitable for injection of living tissue into the blood stream, so that the microcapsules may lodge inside body organs for ongoing metabolism

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therein. While the primary benefit of the smaller
diameter microcapsules of the invention is in in-vivo
uses, the living tissue-containing microcapsules may
also be put to a variety of in-vitro uses.

5   In addition to producing microcapsules containing
living tissue or cells, the present invention may be
used to form microcapsules containing a variety of
other core materials, depending on the intended end
use of the microcapsules.

10   The invention is illustrated further by the
following Examples:

Example 1

         This Example illustrates the formation of small
diameter gel droplets using an electrostatic droplet

15 generator.

         An apparatus as illustrated in Figure 1 was set
up. A 1.5% w/v sodium alginate solution (14) was
placed in a 10 cc syringe (12) to which is attached a
22 gauge stainless steel needle (22) having a 90°

20 bevel outlet. The positive polarity wire (28) was
attached to the metal leur lock section of the needle
and the needle-syringe combination was attached to
the syringe pump (18). A 1.1% calcium chloride
solution (26) was poured into a 4" x 1" petri dish

25 (24) to which was attached the negative polarity wire
(30). The petri dish (24) was positioned so that the
liquid surface therein was 10 mm from the tip of the
needle (22).

         The pulse voltage dial (122) on the adjustment
30 panel (116) was set at 12 KV, the pulse frequency dial
(118) at 20 sec^{-1}, the pulse length dial (120) at 2 m.
sec, and the syringe pump speed at 4 ml/hr. The
syringe pump (18) and droplet generator were both
turned on so that sodium alginate liquid droplets (20)

35 were drawn from the tip (27) of the needle (22) and,
upon entering the calcium chloride solution in the

petri dish (24), calcium alginate gel droplets were formed and were collected therein. The resultant calcium alginate gel droplets were found to be perfectly smooth and spherical and with a mean
5 diameter of 300 (±50 SD) µm.

The syringe needle (22) used in this Example was of the same diameter as was previously used in an air jet syringe wherein a rapidly flowing air stream was used to remove sodium alginate liquid droplets from
10 the tip (27) of the needle (22) using the air jet syringe, the smallest diameter calcium alginate gel droplets attainable had a diameter of 700 µm. The electrostatic procedure described in this Example, therefore, was able to decrease the gel droplet
15 diameter to approximately half this value.

Example 2

This Example illustrates the formation of small diameter gel droplets using an alternative form of droplet generation.

20 The procedure of Example 1 was repeated, except that the apparatus of Figure 2 was utilized, i.e. the negative polarity wire (30) was attached to the needle (22) and the positive polarity wire (28) is attached to the metal ring device (32) which is spaced
25 downwardly from the tip of the needle (22). The centre of the metal ring (32) was positioned 7 mm downwardly from the tip (27) of the needle (22) and an uncharged petri dish (24) was positioned about 5 cm downwardly from the ring assembly.

30 The calcium alginate gel droplets produced by this procedure and collected in the petri dish were observed to be perfectly smooth and spherical and to have a mean diameter of 450 (±65 SD) µm. When the experiment was repeated with the charge reversed, a
35 greater variation of gel droplet diameter was observed with the standard deviation (SD) of gel droplet diameter approximately doubling.

Example 3

This Example illustrates the viability of living tissue after passage through the electrostatic droplet generator.

The procedure of Example 1 was repeated except that islets of Langerhans extracted from the pancreatic tissue of dogs were added to the sodium alginate solution in the syringe in a concentration of 500 islets/2 ml and the calcium chloride solution was replaced by saline, so that gel droplet formation did not occur in this experiment. After passage through the electrostatic droplet generator, 100% of the islets were shown to be viable using Trypan blue staining. All the islets appeared white when viewed under the microscope, there being no evidence of the blue appearance characteristic of dead islets.

Example 4

This Example illustrates the formation of small semi-permeable microcapsules containing islets of Langerhans.

Cultured rat islets of Langerhans (2×10^3 islets in 0.2 ml medium) were suspended uniformly in 2 ml of a 1.5% (2/2) sodium alginate solution (viscosity 51 cps) in physiological saline. Spherical droplets containing islets were produced with an electrostatic droplet generator using the procedure of Example 1 and were collected in 1.5% (w/w) calcium chloride solution. The supernatant was decanted and the gelled spherical calcium alginate droplets, containing islets, were washed with CHES (2-cyclohexylaminoethane sulfonic acid) solution and 1.1% calcium chloride solution.

After aspirating off the supernatant, the gelled droplets were incubated for 6 minutes in 0.05% (w/w) solution of polylysine having a molecular weight of 17,000. The supernatant was decanted and the

polylysine capsules were washed with dilute CHES, 1.1% calcium chloride solution and physiological saline.

The washed polylysine capsules were incubated for 4 minutes in 30 ml of 0.03% sodium alginate to permit
5   the formation of an outer alginate membrane on the initial polylysine membrane, by ionic interaction between the negatively-charged alginate and the positively-charged polylysine.

The resulting microcapsules were washed with
10 saline, 0.05M citrate buffer for 6 minutes to reliquify the inner calcium alginate, and a final saline wash. The microcapsules were found to be perfectly spherical and each to contain from 1 to 2 viable islets. The microcapsules had a mean diameter
15 of 300 (±50 SD) microns and wall thicknesses of 5 μm. The microcapsules were suspended in nutrient medium at 37°C.

The viability of the islets was demonstrated by Trypan Blue staining after the capsule walls were
20 dissociated with heparin.

Example 5

This Example illustrates the formation of small semi-permeable microcapsules containing hepatocytes (liver cells).
25 The procedure of Example 4 was repeated except that fetal mouse or adult rat hepatocytes were added to the sodium alginate solution in amounts of 10^5 hepatocytes/ml of alginate solution and the distance from the tip of the needle to the surface of the
30 calcium chloride solution was decreased to 7 mm. The resulting microcapsules were spherical in appearance and had a diameter of 250 μm (± 50 SD). The presence of viable hepatocytes was demonstrated by Trypan Blue staining and histology, even after more than 4 weeks
35 in culture at 37°C.

Example 6

This Example illustrates the effect of needle
parameters on gel droplet size.

The procedure of Example 1 was repeated, except
5   that a 26 gauge needle having a 22-degree bevel was
used in place of the 22 gauge needle having the
90-degree bevel. The resultant gel droplets had a
diameter of 170 μm (±30 SD), demonstrating the smaller
diameter gel droplets and consequently microcapsules
10   can be formed by using a smaller diameter needle.

In summary of this disclosure, the present
invention provides a novel droplet generation
procedure using electrostatic forces which is
particularly useful in the microencapsulation of
15   living tissue or cells to form small diameter
microcapsules suitable for cardiovascular injection.
Modifications are possible within the scope of the
invention.

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CLAIMS

1. Spherical, smooth and uniform microcapsules suitable for cardiovascular injection into an animal body, characterized by a diameter of less than about 700 μm and containing living tissue or cells.

2. The microcapsules claimed in claim 1 having a diameter of about 150 to about 500 μm.

3. The microcapsules claimed in claim 1 or 2 composed of a biocompatible semi-permeable membrane encapsulating a core of living tissue or cells.

4. The microcapsules claimed in claim 3 characterized in that the living tissue comprises islets of Langerhans.

5. The microcapsules claimed in claim 4 characterized in that the islets are entrapped within a reliquifiable reversible gel material.

6. The microcapsules claimed in claim 5 characterized in that the reversible gel material is calcium alginate.

7. The microcapsules claimed in claim 4 characterized in that the islets are entrapped within reliquified gel material.

8. The microcapsules claimed in claim 7 characterized in that reliquified gel material is aqueous sodium alginate.

9. The microcapsules claimed in any one of claims 1 to 8, characterized by a core comprising one or more viable, healthy, physiologically-active tissue cells capable of on-going metabolism and an aqueous medium of nutrients sufficient to maintain the tissue cells and allow normal metabolism thereof, and a biocompatible semi-permeable membrane surrounding and enclosing the core and being permeable to tissue nutrients and metabolic products produced by the tissue but impermeable to immune system proteins.

10. The microcapsules claimed in claim 9 characterized in that the membrane has a thickness of about 4 to about 6 microns.

11. A method of droplet formation, characterized by the steps of (a) extruding a droplet-forming liquid downwardly from a source at a first location, (b) charging the extruded material with a charge of one polarity, (c) establishing a charge of opposite polarity at a second location spaced vertically below the first location, and (d) providing a difference in voltage between the first and second locations sufficient to form a liquid droplet from the extruded material and draw the same towards the second location.

12. The method claimed in claim 11 characterized in that the first location comprises an axially-downwardly directed electroconductive needle, the second location comprises a recipient medium in which the formed droplets are collected, and the recipient medium comprises a hardening solution to form discrete microcapsules from the formed droplets therein.

13. The method claimed in claim 11 characterized in that the first location comprises an axially-downwardly directed electroconductive needle, second location comprises a ring of electroconductive material surrounding the axis of the needle and being concentric with the axis, a recipient medium is located below the second location to receive and collect the formed droplets therein, and the recipient medium comprises a hardening solution to form discrete microcapsules from the formed droplets therein.

14. The method claimed in any one of claims 11 to 13, characterized in that the voltage difference for droplet formation is applied cyclically in pulses to continuously-extruded droplet-forming liquid to effect continuous production of droplets.

15. The method claimed in claim 14, characterized in that the pulse voltage is from about 1 to about 25 KV, the pulse frequency is about 10 to about 100 sec^{-1}, and the pulse duration is about 1 to about 6 m.sec.

16. Apparatus for droplet formation, characterized by (a) electroconductive extrusion means for extruding a droplet-forming liquid downwardly from a source thereof, (b) means for applying a static electric charge of one polarity to the extrusion means so as to charge the extruded material, (c) means for applying a static electric charge of opposite polarity to an electroconductive body located below the electroconductive extrusion means, and (d) means for varying the voltage between the electroconductive extrusion means and the electroconductive body to exert a discrete droplet-forming force on the droplet-forming liquid extruded from the extrusion means.

17. The apparatus claimed in claim 16, characterized in that the extrusion means comprises a needle and the electroconductive body comprises a recipient medium for collecting the discrete droplets.

18. The apparatus claimed in claim 16, characterized in that the extrusion means comprises a needle and the electroconductive body comprises an electroconductive annular member surrounding the axis of the needle and being concentric therewith.

19. The apparatus claimed in any one of claims 16 to 18, characterized in that the means for varying the voltage comprises voltage pulse generating means for generating continuous voltage pulse above a predetermined threshold voltage level.

20. The apparatus claimed in claim 19, characterized in that the voltage pulse generating means is capable of a pulse voltage of about 1 to about 25 KV, a pulse frequency of about 10 to about 100 sec^{-1} and a pulse duration of about 1 to about 6 m.secs.
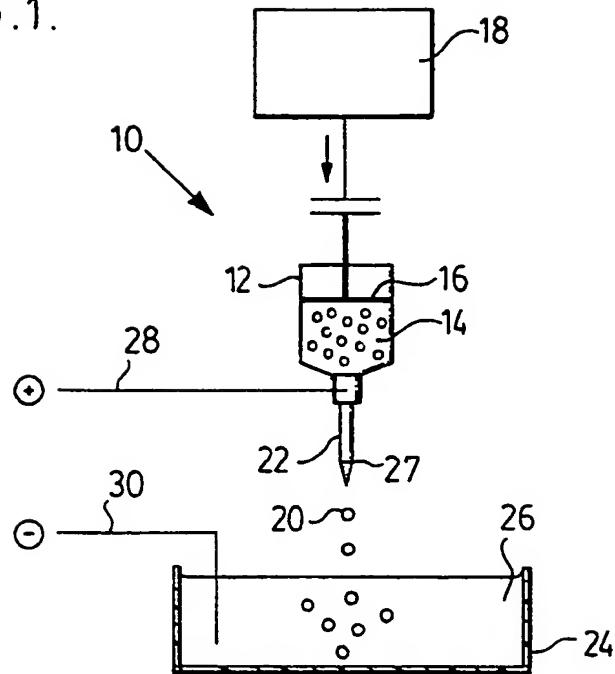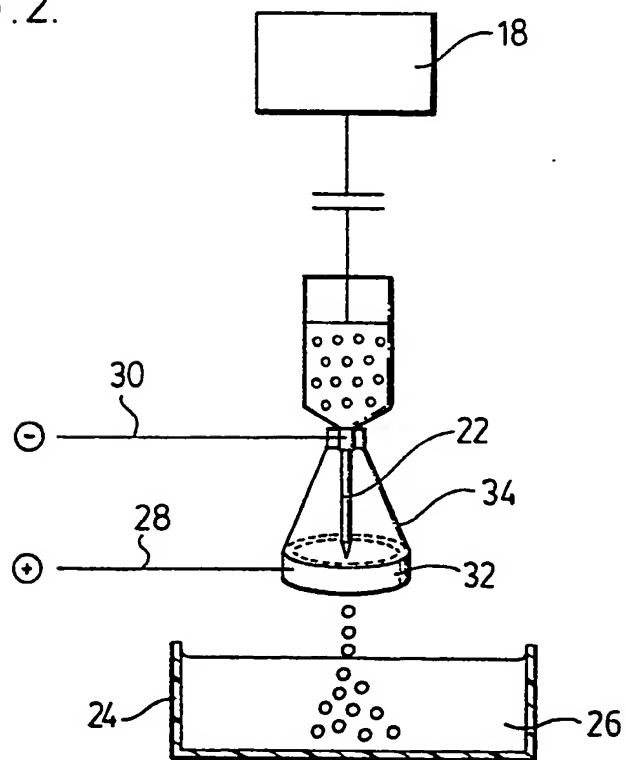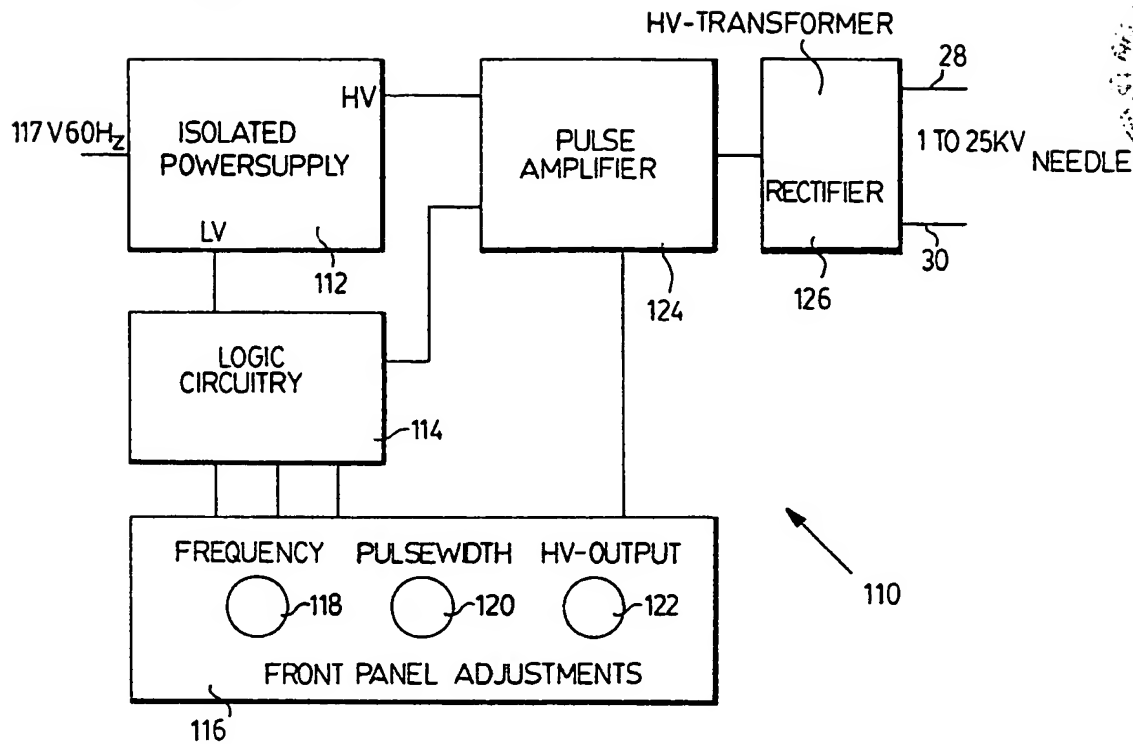
FIG.1.

10

18

12   16

28   14

22   27

30   20   26

24

FIG.2.

18

30   22

28   34

32

24   26

FIG. 3.

HV-TRANSFORMER

117 V 60Hz → ISOLATED POWERSUPPLY — HV → PULSE AMPLIFIER → RECTIFIER → 28 — 1 TO 25KV NEEDLE — 30

112

LV

LOGIC CIRCUITRY

114

124

126

110

FREQUENCY   PULSEWIDTH   HV-OUTPUT

118   120   122

FRONT PANEL ADJUSTMENTS

116

FIG. 4.

PULSE FREQUENCY

PULSE LENGTH

VOLTAGE (Kv)

PULSED VOLTAGE

BASELINE VOLTAGE

TIME (m sec)